The impact of platelet-derived growth factor on closure of chronic tympanic membrane perforations: a randomized, double-blind, placebo-controlled study

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Abstract: Objective: Patients with tympanic membrane (TM) perforations often suffer from infections, and repetitive topical treatment may be required. These infections can be prevented by permanent closure of the TM perforation. Different surgical treatment options have been described, but non-invasive techniques may be preferred as they carry less risk than surgery. One non-invasive approach is to induce wound healing by application of growth factors. The effect and clinical utility of applying topical platelet derived growth factor (PDGF) for decrease of size and closure of chronic TM perforations is evaluated. Study design: Prospective, randomized, placebo controlled, double blind study Setting: Tertiary referral center. Patients: Twenty patients suffering with chronic suppurative otitis media without cholesteatoma for more than 3 months. Intervention: Topical treatment with PDGF or placebo applied weekly to the TM for 6 weeks. Main outcome measures: Success rate, defined as a reduction of perforation size of 50% or more to determine relative changes of the perforation size; effect of initial size and location of TM perforation on success rate, and air and bone conduction thresholds to determine air-bone gap (ABG) measured before treatment. Results: Randomization made matching pre-treatment perforation size of the two study groups impossible, and the initial rate perforation/TM was significantly smaller in the PDGF group. No difference between the two groups was found for perforation/TM $< 10\%$. However, success rate did not differ significantly between the two groups (Power=0.8), and the effect of PDGF was found to be small (-2%, +-49% STD). Initial size and position of the TM perforation were not significant factors determining success. Mean ABG for the frequencies of 0.5, 1, 2, and 4 kHz was 22.5 dB. Conclusion: The topical application of PDGF as an office treatment for chronic otitis media is not a favourable alternative to surgery.

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The impact of platelet-derived growth factor on closure of chronic tympanic membrane perforations

- a randomized, double-blind, placebo controlled study

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Abstract

Objective: Patients with tympanic membrane (TM) perforations often suffer from infections, and repetitive topical treatment may be required. These infections can be prevented by permanent closure of the TM perforation. Different surgical treatment options have been described, but non-invasive techniques may be preferred as they carry less risk than surgery. One non-invasive approach is to induce wound healing by application of growth factors. The effect and clinical utility of applying topical platelet derived growth factor (PDGF) for decrease of size and closure of chronic TM perforations is evaluated.
Study design: Prospective, randomized, placebo controlled, double blind study
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Main outcome measures: Success rate, defined as a reduction of perforation size of 50% or more to determine relative changes of the perforation size; effect of initial size and location of TM perforation on success rate, and air and bone conduction thresholds to determine air-bone gap (ABG) measured before treatment.
Results: Randomization made matching pre-treatment perforation size of the two study groups impossible, and the initial rate perforation/TM was significantly smaller in the PDGF group. No difference between the two groups was found for perforation/TM < 10%. However, success rate did not differ significantly between the two groups (Power=0.8), and the effect of PDGF was found to be small (-2%, +-49% STD). Initial size and position of the TM perforation were not significant factors determining success. Mean ABG for the frequencies of 0.5, 1, 2, and 4 kHz was 22.5 dB.
Conclusion: The topical application of PDGF as an office treatment for chronic otitis media is not a favourable alternative to surgery.

Introduction

Tympanic membrane (TM) perforations have an estimated incidence of 1 and 8.6% (1, 2). In some patients, TM perforations heal spontaneously (3, 4), or healing can be induced with minimally invasive surgery to create fresh wounds at the margin of the perforation. The healing process includes epithelial migration, increased fibroblastic activity and vascular proliferation. However, these reparative mechanisms seem to become deactivated in some patients for unknown reasons and the result is a persistent TM perforation. The sequela can manifest as recurring infections that make repetitive topical treatment necessary and / or conductive hearing loss. To prevent recurring infections, patients need to avoid water from getting in the ear and are restrained from swimming. Such patients often seek advice concerning permanent closure of the TM perforation.

One approach used to close a persistent TM perforation is to provide stromal support for epithelialisation. This can be achieved surgically. Different graft materials such as fascia, fat, perichondrium or cartilage are used as stromal support. Surgery has some drawbacks. Beside the surgical risks, such as bleeding, infections and possible damage of the ossicular chain with hearing impairment, the success rate for permanent closure of the TM perforation over the long term is about 80% (5, 6). Furthermore, surgery is not accessible to all patients because of its costs and availability.

Another approach is to induce cellular replication and migration resulting in partial or total closure of a TM perforation. Whether or not the size of a TM perforation is a predictor of success for the surgical treatment is still a matter of debate. However, in a prospective trial conducted by the Royal College of Surgeons of England, it was clearly demonstrated that small perforations (less than 50% of the TM size) had a higher success rate (7) than did larger ones. Therefore, even if a complete closure of the TM is not achieved by this form of treatment, a preoperative intervention resulting in a reduction of the size of the TM perforation remains desirable.

Several growth factors are involved in wound healing such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), transforming growth factor-alpha (TGF-alpha), and platelet derived growth factor (PDGF) (8). These growth factors act chemotactically and induce proliferation of the cells involved in wound healing (9, 10).

One of these growth factors is PDGF, a heterodimeric amino acid of approximately 30-kD of two distinct but homologous polypeptide chains. It is released from platelet c-granules, endothelial cells, fibroblasts, smooth muscle cells, and macrophages (11). Platelet derived growth factor is a potent mitogen for connective tissue cells and fibroblasts. It enhances the production of fibronectin and hyaluronic acid, and induces growth factor-beta, which initiates collagen production with fibroblasts (12). Platelet derived growth factor is used clinically and improves repair of a chronic cutaneous ulcer after stimulation of cell replication after 8 weeks (13). Its application is safe if used in small doses. No toxic reaction occurred in a phase I/II trial by Robson et al. investigating the effect of PDGF on chronic pressure ulcers (14). No effect on epithelial dysplasia could be shown in an animal study using hamsters (15), however application of more than 45g of Regranex® Gel (PDGF 0.01%, Janssen-Cilag, Belgium), has been reported to induce malignancy (16).

The effect of PDGF on TM closure has been investigated in animal models with contradictory results, but not in humans. While Soumekh et al. (17) found no statistical difference in their chinchilla model using platelet releasate, Yeo et al. (18) describes a faster closure of TM perforations in a rat model undergoing a similar treatment regimen.

Our randomized, double blind, placebo controlled prospective study evaluates the benefit of topical PDGF in closure of chronic TM perforations in human. As in a randomized study design matching of the initial perforation size for treatment and control group is not possible, we investigated a) not only closure rate, but success rate defined as a reduction of perforation size by 50% or more, and b) influence of initial size of perforation on the outcome. Furthermore, we studied c) influence of position of perforation on success, and d) hearing threshold measured before treatment.

Material und Methods

Patients suffering with suppurative chronic otitis media without cholesteatoma for more than 3 months were included in the study. Exclusion criteria were acute infection of the ear, traumatic TM perforations, age < 18, pregnancy or any known allergic reactions to PDGF. After obtaining informed consent, the patients were randomized in a block scheme, 10 to the placebo group, and 10 to the intervention group. They were investigated between May 2008 and August 2010. Seven patients were female, 13 were male. The mean age was 43.4 years (range 24 to 74 years).

Before treatment, at every check-up investigation, and at the end of the treatment, the size of the TM perforation was photographed with a 0° optic. The area of the TM perforation relative to the size of the whole TM was determined and compared at the beginning (Pre-treatment, PreTret) and at the end of the investigation (Post-treatment, PostTret) using ImageJ software (NIH, Bethesda, MD, USA). The closure of the perforation was calculated [(PostTret – PreTret)/PreTret]. The absolute size of the TM perforation was estimated. As there was no reference for size on the picture taken, we assumed a vertical diameter of the TM as measured along the axis of the manubrium of 9.5mm and a horizontal diameter of the TM of 8.5m (19).

Treatment consisted of the following steps and was performed as an office procedure with the patient seated in an upright position: Local anaesthesia using a small piece of cotton soaked with 10 % Lidocaine hydrochloridum anhydricum (Xylocain, AstraZeneca, USA) was administered in the external ear canal on the remaining TM. The perforation edge was stripped under a microscope through the ear canal using a biopsy forceps to create a fresh wound surface. According to the randomisation scheme, a paper patch soaked with approximately 0.1 ml PDGF (Regranex® Gel 0.01%, Janssen-Cilag, Belgium), or placebo was administered on the TM such that it covered the perforation completely. This patch was changed every 7 days for 6 weeks or until the perforation was closed completely.

The placebo was specifically manufactured for this study and consisted of purified water (94.05g/100g), Nipacombin (a preservative) in Propylene glycol 10% (a solvent) (0.5g/100g), Chlorocreasol (a preservative that mimics the smell of Regranex ®) (0.05g/100g), Carboxymethylcellulose sodium (an additive for gelation to achieve the desired viscosity) (5.4g/100g). This composition resulted in an almost identical viscosity compared to Regranex ® Gel 0.01%. The smell, appearance and package of the two substances were identical.

If the TM perforation did not close after 6 weeks, then a surgical closure was offered to the patient to be performed three months after the end of the intervention.

Hearing thresholds were measured using pur- tone audiometry for air conduction at 0.125, 0.25, 0.5, 1, 2, 4, and 8 kHz and for bone conduction (BC) at 0.5, 1, 2, and 4 kHz before treatment. The air- bone gap (ABG) was calculated for the frequencies of 0.5, 1, 2, and 4 kHz.

To determine the number of patients required for this study, a power analysis was performed resulting in a total of 20 patients required to show a significant difference. In our study a reduction of perforation size of 50% or more was considered clinically significant.

The study was approved by the ethical committee and was performed according to the Declaration of Helsinki (20). Independent sample t-test analyses were performed to calculate the statistical difference for the two groups.

Results

Upon initial enrolment in the study, all patients reported recurrence of acute infections and some described hearing deterioration. The perforation was located on the right TM in 11 patients and on the left TM in 9 patients. The characteristics of the patients treated with either PDGF or placebo are shown in Table 1. No severe complications such as infections, hearing impairment, tinnitus, vertigo, bleeding, or hyperkeratosis were observed during treatment. Six patients (3 patients in the PDGF, and 3 patients in the placebo group) did not tolerate stripping the perforation edge of the entire circumference due to discomfort despite careful application of local anaesthesia. In these patients, the perforation edge could only be partially (50 to 80%) stripped.

The perforations before and after treatment are shown in Figure 1a for PDGF and in 1b for placebo. Complete closure of the perforation was seen in one patient in each group (10%) and did not differ significantly between the groups ($p = 1$). The perforation was larger after the intervention in 4 patients in the PDGF group and in 2 patients in the placebo group. The estimated horizontal and vertical diameters are shown in Table 1. The reduction of the ratio perforation/TM was found to be 31% (+- 36% STD) and -2% (+-49% STD) for the placebo and the PDGF groups, respectively. These differences were not statistically significant ($p = 0.097$). A post hoc power analysis was performed and showed a power of 80% to detect a clinically significant closure (as defined by 50% or more decrease of the size) of the TM perforation (Figure 2).

The ratio of the TM perforation/TM before the intervention was found to be larger in the placebo group (22%; SDT +- 14%) compared to the PDGF group (10%; SDT +- 8%) and showed statistical significance ($p = 0.022$).
A pre-treatment ratio perforation/TM < 10% was found in 6 patients in the PDGF group and in 3 patients in the placebo group, and showed no statistical difference ($p=0.84$). In patients with a pre-treatment ratio perforation/TM > 10%, the ratio was significantly smaller ($p=0.016$) in the PDGF group ($n=4$) compared to the placebo group ($n=7$). Success rate did not differ significantly in patients with pre-treatment ratio perforation/TM < 10% ($p=0.21$) or > 10% ($p=0.39$).

The perforation was located in the lower anterior quadrant in 9 patients of the PDGF group and in 6 patients in the placebo group. In the placebo group, the perforation was also found in the anterior superior quadrant in 2 patients, in the posterior inferior quadrant in 1 patient, and in the posterior superior quadrant in 1 patient. In 1 patient of the PDGF group, the perforation was located in the posterior inferior quadrant. The position of the perforation was not a significant factor for success ($p = 0.29$).

The individual and mean pure-tone air- and bone-conduction thresholds before treatment are displayed in Figure 3a and b for the PDGF and the placebo group. Mean pure tone average for four frequencies (0.5, 1, 2, 4 kHz) for BC was 17.5 dB for the PDGF group and 12 dB for the placebo group. Mean pure tone average for air conduction for the seven frequencies (0.125, 0.25, 0.5, 1, 2, 4, 8 kHz) was 50 dB for the PDGF group and 38.5 dB for the placebo group.

Discussion

Chronic TM perforations have a smaller spontaneous healing rate compared to acute TM perforations. Three possible requisites for healing of TM perforations are discussed in the literature.

Some authors state that epithelialisation of the edges of a longstanding perforation may constrain spontaneous healing by preventing vascular proliferation (1, 2). As proliferation of a subepidermal connective tissue layer lags behind proliferation of the epithelial layer, rolled epithelial edges may develop and closure of the TM perforation is inhibited. Hakuba et al. (21) state that disruption of the rolled epithelial edge of the TM perforation is required to induce the growth of epithelial cells. Although we intended to strip the perforation edge circularly, 6 patients (30%) did not tolerate this intervention due to discomfort and only about 50 to 80% of the perforation was successfully stripped. This drawback reflects the difficulty of transferring a method working in the laboratory environment to the clinic. The patients were evenly distributed between the two groups (3 patients in the placebo group and PDGF group, respectively). Therefore, the incomplete stripping of the perforation edge did not affect the comparison of the two groups. However, it may be a reason for the low success rate in our study, which was 10% in both groups compared to the 92% of Hakuba et al. (21) using bFGF. There must be additional factors for the lower success rate in our study as Lee et al. (22) did not strip the perforation edge and reported a success rate of 80% in a chinchilla model. He states that the excision of the margin of the TM perforation is not essential for EGF efficacy.

A second factor for closure of a TM perforation is the need for a guiding structure for epithelial proliferation. In contrast to wound healing in other locations of the body, no granulation tissue to act as a guiding structure develops, but replication of epithelium cells occurs - triggered by vascular proliferation. We used a paper patch soaked with PDGF or placebo to cover the TM perforation. Paper patches are considered to be an adequate material as a guiding structure (23) and no inflammatory reaction to the material was seen clinically. Other materials such as MeroGel (24), hyaluronic acid (25), atelocollagen (21), and Gelfoam (18, 22) have also been used. We did not compare different guiding structures in our study. A different material might have resulted in more favourable results. Materials such as atelocollagen can retain PDGF in its micropores and release it slowly and a longer working time of PDGF can be achieved. However, the need of a structural scaffold is challenged by Lee et al. (22) who reports a high success rate without placing any guidance structure for epithelium migration. As they used a chinchilla model, absolute size of the TM perforation was much smaller compared to human, who might benefit from a guiding structure.

A third factor is the need to trigger the proliferation of the epithelial cells on the edge of the TM perforation. However, application of PDGF did not result in a higher closure rate in our study. Platelet derived growth factor was chosen because it induced earlier closure of TM perforations in rats compared to untreated TM perforations (18). Application occurred over a period of 42 days in our study, which seems to be a sufficient length of time, according to Robson et al. (14), who found an effect of PDGF after treatment of 28 days in 20 patients with a chronic pressure ulcus. However, he only found significance in patients treated with 100 mcg/ml (0.01%), which is the concentration used in our study, and no significance 10 and 1 mcg/ml. As the action time of PDGF decreases gradually after its application, an application more often than once per week might have been beneficial. But it might also have interfered with the patient's compliance as it is unpractical for many patients to be absent from work more than once a week for several weeks.

Other growth factors have been investigated with contradictory results. Lee et al. (22) reported a success rate of 80% with EGF compared to 20% with placebo in chinchilla, while Soumekh et al. (17) did not report an advantage of platelet derived releasate in his chinchilla model. Other studies have reported a faster closure of TM perforations using bFGF in guinea pigs (26) or rats (27, 28). One clinical study (21) using bFGF showed a complete closure in 92%. In contrast to our study, no control group was used and the mean perforation size of 14.4% of the TM was rather small. Additionally, not only patients with TM perforation due to chronic infections, but also patients with traumatic TM perforations were included in the

study. Therefore, a straight forward comparison to our results is not possible. In a double-blind placebo controlled trial, Ramsey et al. (29) found no advantage of EGF in closure of a chronic TM perforation after a mean follow up of 2.6 months.
In a randomized study design like in our study, matching the perforation size of two treatment groups is not possible and differences between the groups can occur. These differences can only be seen after the experimental part of a study during data analysis. In our study, the size of the pre-treatment ratio perforation/TM is significantly smaller in the PDGF group compared to the placebo group. To prevent a bias arising from this difference, we did not only analyse closure rate of the TM perforation, but the relative change of perforation size as well. For this purpose, we defined success rate as a 50% reduction of the ratio perforation/TM. The post hoc power analysis showed a power of 80% for identifying a 50% or greater decrease of the TM perforation.
As small perforations might heal faster (1, 2) while large perforations of > 50% of the total TM result in delayed TM closure (7, 30), patients with small perforations were analysed additionally. The pre-treatment ratio perforation/TM for small perforations did not differ significantly between PDGF and placebo group. The two patients with complete closure of the TM perforation, in our study, both had small perforations. Success rate and closure rate between PDGF and placebo group did not differ significantly for small perforations.
The smaller size of the TM perforation in patients in the PDGF group did not result in a higher overall success rate. Clinically, there was no benefit from PDGF, overall, in patients with small, or in patients with large TM perforations. Possible differences between groups seem to be small if at all.

The location of the perforation differed between the PDGF and the placebo groups, but this did not influence the success rate, which agrees with the findings of Orji et al. (30) who investigated 53 patients with traumatic TM perforations. Some authors consider a perforation in the posterior anterior quadrant as having the highest risk of persistence (31).

Conclusion

The use of PDGF in our investigation was of no advantage compared to a placebo. The sample size was large enough to exclude TM perforation size closure of 50% or more (power=0.8). Furthermore, application is time consuming and uncomfortable to many patients, despite local anaesthesia. The topical application of PDGF should not be considered as a favourable alternative to surgery, based upon these results.

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Tables and Figures

Table 1

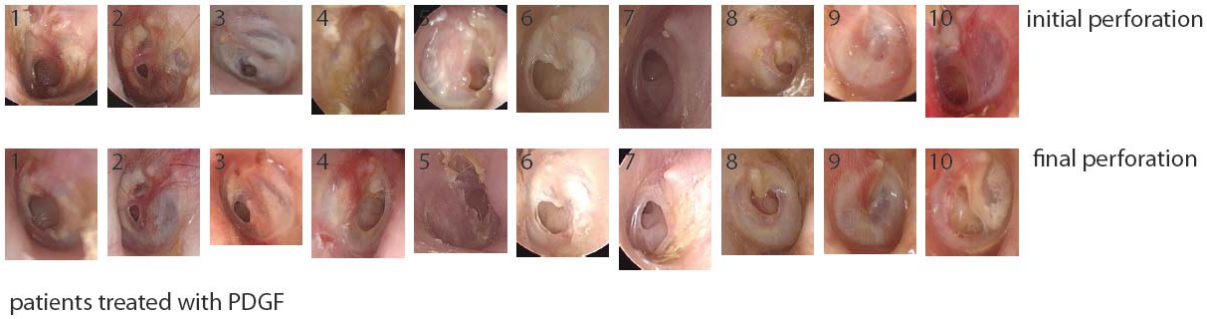
Comparison of the characteristics of the patients of the two groups, the size of the perforation before and after intervention and the ABG (air-bone gap) before and after intervention.

	PDGF	Placebo
mean age (years) range (years)	45.5 (24 to 65)	41.3 (24 to 74)
gender (male / female)	5 / 5	8 / 2
side (right / left)	5 / 5	6 / 4
relation perforation size : TM before intervention	0.1	0.22
relation perforation size : TM after intervention	0.1	0.18
estimated diameter of TM before intervention (mm) vertical / horizontal	2.8 / 2.35	4.7 / 3.7
estimated diameter of TM after intervention (mm) vertical / horizontal	2.8 / 2.3	4.0 / 3.2
complete closure (n)	1	1
mean ABG before treatment 0.5, 1, 2, 4 kHz (dB)	23.5	21.5

Figure 1A and B

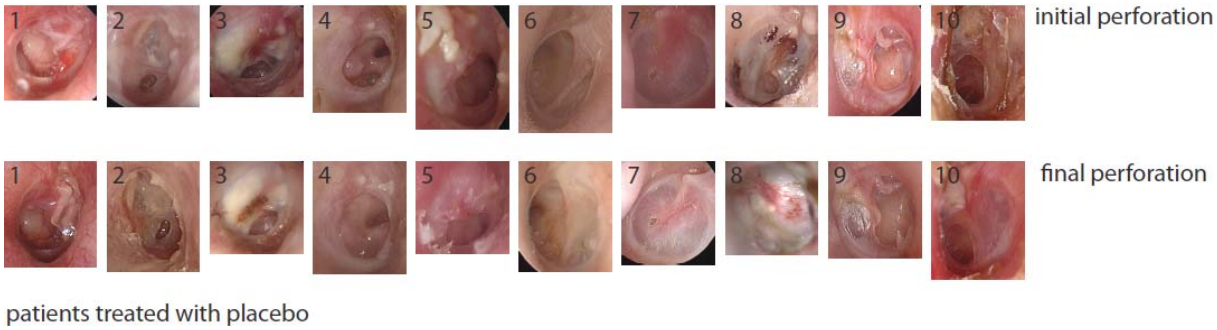
The photograph of the perforations before (upper row) and after treatment (lower row) are shown in for the PDGF group (1a) and the placebo group (1b). A complete closure was seen in patient 9 in the PDGF group and in patient 8 in the placebo group.

A



patients treated with PDGF

B



patients treated with placebo

Figure 2

Decrease of TM perforation after intervention for the placebo group (○) and the PDGF group (●). The TM perforation closed completely in one patient in each group. A > 50% decrease of the TM perforation was found in two patients in the placebo group and in one patent in the PDGF group. These differences were not significant (p = 0.097).

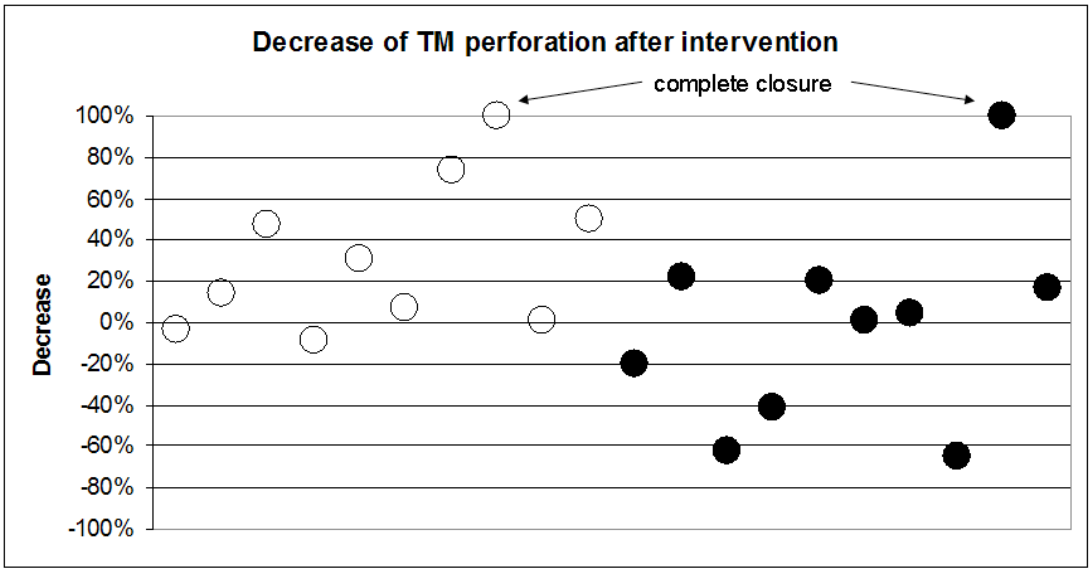
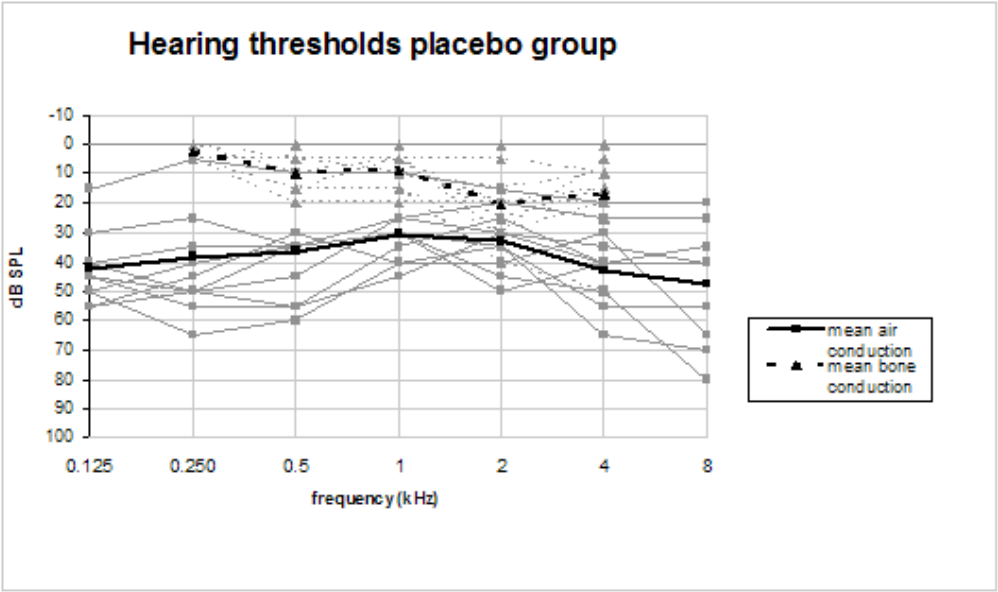


Figure 3

The individual (grey) and mean (black) hearing thresholds before treatment are shown for air- and bone conduction for the placebo group in a) and for the PDGF group in b). The dotted line represents bone conduction thresholds, the solid line represents air conduction thresholds.

A)



B)